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Biochemistry

Phosphorylation of ribosomal protein S6 on serine after microinjection of the Abelson murine leukemia virus tyrosine-specific protein kinase into *Xenopus* oocytes

(tyrosyl-protein kinases/growth control)

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ABSTRACT **Phosphorylation of ribosomal protein S6 in NIH 3T3 fibroblasts is dependent on the presence of serum, but after transformation of these cells by Abelson murine leukemia virus (Ab-MuLV), S6 remained highly phosphorylated on serine residues either in the absence or the presence of serum. To investigate whether S6 phosphorylation in this system was a consequence of the action of the Ab-MuLV tyrosine-specific protein kinase, purified Ab-MuLV kinase made in *Escherichia coli* was microinjected into *Xenopus* oocytes and was observed to cause a 7- to 15-fold increase in the phosphorylation of S6 on serine residues. Two-dimensional phosphopeptide maps of S6 phosphorylated in Ab-MuLV-transformed NIH cells in the absence of serum were identical to those of S6 isolated from normal cells grown in the presence of serum. In addition, S6 from oocytes injected with Ab-MuLV kinase yielded an S6 phosphopeptide map indistinguishable from that of serum-stimulated NIH 3T3 cells, whereas S6 from control oocytes lacked several phosphopeptides. Ab-MuLV kinase did not phosphorylate S6 directly *in vitro*, and microinjection of a mutant Ab-MuLV protein lacking kinase activity had no effect. These results indicate that the Ab-MuLV kinase interacts with a cellular pathway to enhance S6 phosphorylation by directly or indirectly activating an S6 protein kinase and/or inactivating an S6 protein phosphatase.**

Tyrosine-specific protein kinases are encoded by the transforming gene products of several retroviruses (1–9). Similar kinase activities are found also in association with the membrane receptors for epidermal growth factor (EGF) (10), platelet-derived growth factor (PDGF) (11), insulin-like growth factor (12, 13), and insulin (14). These findings have suggested that phosphorylation of proteins on tyrosine residues is involved in the control of cell growth by transforming gene products as well as normal cellular growth factors. However, physiologically important substrates for these enzymes have yet to be identified in any of these systems. Although all of the above protein kinases appear to be specific for tyrosine residues *in vitro*, the addition of epidermal growth factor (15), platelet-derived growth factor (16), or insulin (15, 17, 18) to responsive cells also results in increased phosphorylation of certain proteins on serine residues. Similarly, in cells transformed by either Rous sarcoma virus (RSV) or Abelson murine leukemia virus (Ab-MuLV), both of which encode tyrosine-specific protein kinases, an increase in protein-bound phosphoserine has been observed (19, 20). Among these phosphoseryl-proteins, ribosomal protein S6 is of particular interest because its phosphorylation is correlated with growth-promoting stimuli in a wide variety of systems, including serum- and growth factor-induced cell proliferation (15, 21, 22), *Xenopus* oocyte maturation or fertilization (23–25), regenerating rat liver (26), viral infection (27), and viral transformation (20). In the case of cells transformed with a temperature-sensitive transformation mutant of RSV, enhanced phosphorylation of S6 on serine residues is also temperature sensitive (20).

The widespread correlation between S6 phosphorylation and the growth-promoting actions of such a diverse group of agents suggests that S6 phosphorylation plays an important role in regulating growth. The precise function of S6 phosphorylation in protein synthesis or cell growth has not yet been elucidated, but S6 can be crosslinked to synthetic mRNAs on the ribosome, implying a role in mRNA binding (28). Phosphorylation of S6 has been shown to affect both the binding and translation efficiency of poly(A,U,G) in a reconstituted protein synthesis system (29). Ribosomes containing the maximally phosphorylated derivative of S6 (4–5 mol of phosphate per mol of S6) are preferentially incorporated into new polyribosomes following serum stimulation of 3T3 cells (15, 30), suggesting that early growth-promoting mRNAs may be selectively translated by phosphorylated ribosomes. This idea has been supported by studies in *Drosophila* cells subjected to heat shock, which causes rapid S6 dephosphorylation and results in ribosomes that are unable to translate specific mRNAs *in vitro* (31, 32). These results indicate that S6 phosphorylation may play a role in recruitment of specific mRNAs and enhanced synthesis of certain proteins.

In normal Swiss mouse 3T3 fibroblasts, phosphorylation of S6 is dependent on the presence of exogenous serum or growth factors in the medium (15). In this report, we demonstrate that in Ab-MuLV-transformed NIH 3T3 fibroblasts (cell line ANN-1), S6 is constitutively phosphorylated in the presence or absence of serum. Little is known about the enzymology of S6 phosphorylation, but the fact that growth-promoting agents associated with tyrosine-specific protein kinases enhance S6 phosphorylation on serine residues implies that such tyrosyl-protein kinases are capable of regulating the enzymes that control S6 phosphorylation. One approach to the study of this interaction is to introduce a tyrosine-specific protein kinase into a normal cell. A suitable cell for such experiments is the *Xenopus* oocyte, which can be injected with 0.1-μl volumes. The phosphorylation of *Xenopus* S6 has been shown to be increased during oocyte maturation induced by either progesterone or insulin (24, 25) and in response to microinjection of either the maturation-promoting factor (24) or the transforming protein of RSV (33).

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Abbreviations: MuLV, murine leukemia virus; Ab-MuLV, Abelson MuLV; Mo-MuLV, Moloney MuLV; RSV, Rous sarcoma virus.

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MATERIALS AND METHODS

Isolation of oocytes, microinjection, radiolabeling, and preparation of oocyte phosphorylated ribosomes were carried out as described (25, 34). NIH 3T3 fibroblasts and ANN-1 fibroblasts were grown in Dulbecco's modified Eagle's medium (DME medium) with 10% calf serum. The night before being labeled, cells were plated with 10% serum at 1×10^6 cells per 10-cm dish. The cells were then washed two or three times in DME medium, incubated for 4 hr, and washed twice in phosphate-free DME medium—all in the absence of serum. Labeling was carried out in 3 ml of phosphate-free DME medium containing 3 mCi (1 Ci = 37 GBq) of $^{32}P_i$ (New England Nuclear) for 2 hr with or without 10% dialyzed calf serum for the last hour. The cells were washed in ice-cold phosphate-buffered saline and were lysed in 2.0 ml of 50 mM Pipes, pH 7.5/5 mM $MgCl_2$/5 mM KCl/50 mM NaF/40 μM EDTA/1% (wt/vol) deoxycholate/1% (wt/vol) Triton X-100. Extracts were clarified by microcentrifugation (13,000 $\times$ *g* for 10 min) and layered over 2.0 ml of 1.6 M sucrose/50 mM Pipes, pH 7.5/5 mM $MgCl_2$/0.5 M KCl/40 μM EDTA, and the ribosomes were pelleted by centrifugation (160,000 $\times$ *g* at 2°C for 4.5 hr).

For identification of S6 and determination of the extent of its phosphorylation, the ribosomes were suspended and the ribosomal proteins were extracted, precipitated, and subjected to two-dimensional gel electrophoresis by the procedure of Thomas *et al.* (15) in the presence of carrier ribosomal proteins from *Xenopus* oocytes (unphosphorylated S6) and unfertilized eggs (phosphorylated S6). The labeled S6 was localized by autoradiography, and the ribosomal proteins were visualized by staining with Coomassie blue. For phosphopeptide analysis, the ribosomes were suspended in H_2O, heated to 95°C for 3 min in electrophoresis sample buffer (0.07 M Tris, pH 6.8/11% glycerol/3% NaDodSO$_4$/5% 2-mercaptoethanol/0.002% bromophenol blue), and subjected to electrophoresis through a 12.5% NaDodSO$_4$/polyacrylamide gel. S6 was located by autoradiography, eluted, precipitated with trichloroacetic acid, and hydrolyzed with trypsin under the conditions described by Martin-Perez *et al.* (35) or with chymotrypsin. The phosphopeptides were resolved by electrophoresis on 20 $\times$ 20 cm thin-layer cellulose sheets (Kodak) for 45 min in the first dimension (pH 1.5, 760V) and 50 min in the second dimension (pH 3.5, 760V). Phosphoamino acid analysis was carried out as described (1, 3).

The Ab-MuLV-encoded protein kinase was purified from *Escherichia coli* carrying a vector designed to allow expression of sequences, termed v-*abl*, that encode the Ab-MuLV kinase activity. Construction of the vector will be published in detail elsewhere. Briefly, the vector pCS4 (36) contains the P_R promoter of bacteriophage λ, a ribosome-binding sequence, and an ATG codon followed by 0.24 kilobase pair of sequences coding for the small tumor antigen of simian virus 40. A temperature-sensitive *c*I gene of bacteriophage λ is also present so that transcription is repressed at 30°C and induced at 42°C. To this vector we added 1.2 kilobase pairs of v-*abl* sequences coding for a specific NH_2-terminal region of the Ab-MuLV-encoded kinase [from the *Hin*cII site at the Moloney murine leukemia virus (Mo-MuLV)/Ab-MuLV DNA junction to the next *Pst* I site]. This construct produces a fusion protein consisting of 80 amino acids of the small tumor antigen and 404 amino acids of the kinase but lacks any sequences derived from the *gag* region of Mo-MuLV. The function of the small tumor antigen is to increase the amount of protein produced in bacteria. For control experiments, a fusion protein was constructed with the same vector, but 53 amino acid residues of the kinase portion were deleted (terminating at the first *Sac* I site in the v-*abl* gene), resulting in a product that was kinase defective. Purification of the active kinase (unpublished data) involves a combination of conventional chromatography (DEAE-cellulose, hydroxylapatite, Affi-Gel blue), HPLC, and use of a column coupled to a monoclonal antibody directed towards phosphotyrosine (37). This column adsorbs many of the phosphotyrosyl proteins present in Ab-MuLV-transformed cells (37). Since the Ab-MuLV kinase undergoes autophosphorylation on tyrosine, the kinase binds to the monoclonal column and can be eluted by the addition of *para*-nitrophenyl phosphate, a phosphotyrosine analog. At 100 μM ATP and 1 mg of angiotensin II (used as an artificial substrate) per ml, the specific activity of the purified enzyme at 30°C was $\approx$2.0 μmol/min per mg. For microinjection experiments in which partially purified preparations were used (see Fig. 2), the bacteria were lysed by sonication, extracts were clarified by centrifugation (190,000 $\times$ *g* for 45 min), subjected to batchwise chromatography on DEAE-cellulose (20–100 mM NaCl, pH 8.2), concentrated in an Amicon stirred cell (YM10 membrane), and finally dialyzed against 40% ethylene glycol/1 mM dithiothreitol/0.1 mM EDTA/50 mM Pipes, pH 7.5.

RESULTS

Previously, it had been reported that transformation of cells by RSV results in the phosphorylation of S6 becoming independent of the presence of serum (20). To determine whether Ab-MuLV has a similar effect, NIH 3T3 fibroblasts and Ab-MuLV-transformed fibroblasts were serum-starved, labeled with $^{32}P_i$, and then incubated for 1 hr in the presence or absence of serum. Ribosomes were isolated, and the ribosomal proteins were subjected to two-dimensional gel electrophoresis by a system developed for ribosomal proteins (15). In this system, the phosphorylated derivatives of S6 can be resolved into discrete spots designated a–e, corresponding to 0–5 mol of phosphate per mol of S6, respectively (15). S6 phosphorylation was virtually undetectable in normal NIH 3T3 cells in the absence of serum but was stimulated by addition of 10% serum (Fig. 1 *A* and *B*). In ANN-1 cells, S6 was phosphorylated in the absence of serum, indicating that expression of the Ab-MuLV transforming gene product causes S6 phosphorylation to occur constitutively (Fig. 1 *C* and *D*). The phosphorylated species in ANN-1 cells con-

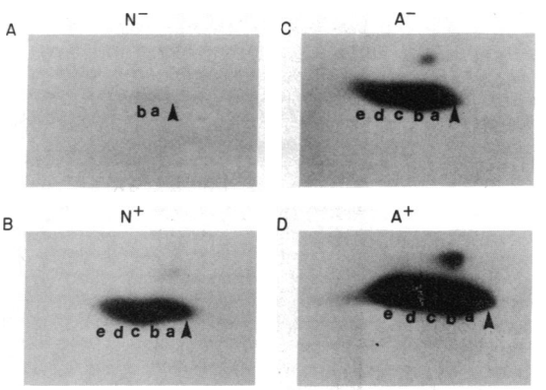

FIG. 1. The effect of transformation by Ab-MuLV on S6 phosphorylation in the presence or absence of serum. NIH 3T3 cells and ANN-1 cells were labeled with $^{32}P_i$ in the presence or absence of serum as described. Ribosomes were isolated, and the ribosomal proteins were subjected to two-dimensional gel electrophoresis in the presence of oocyte (unphosphorylated S6) and egg (phosphorylated S6) ribosomal carrier protein. The arrowhead marks the position of unphosphorylated S6, and the letters a–e refer to S6 derivatives with increasing numbers of phosphate groups, as judged by the Coomassie blue staining pattern of the carrier protein. (*A*) NIH 3T3 fibroblasts in the absence of serum. (*B*) NIH 3T3 fibroblasts in the presence of 10% serum. (*C*) ANN-1 cells in the absence of serum. (*D*) ANN-1 cells in the presence of 10% serum.

tained $^{32}P_i$ in all derivatives of S6. No significance is attached to the slightly greater level of total S6 phosphorylation after serum treatment of ANN-1 cells (Fig. 1*D*) because this was not apparent in other experiments of this type.

After transformation of cells by retroviruses, a multitude of cell parameters are altered (38). To determine whether the constitutive phosphorylation of S6 in ANN-1 cells was an effect of the Ab-MuLV-encoded kinase, we microinjected into *Xenopus* oocytes a homogeneous preparation of the kinase domain of the Ab-MuLV transforming protein. Following microinjection, there was a significant increase in S6 phosphorylation relative to uninjected oocytes or oocytes injected with the kinase buffer (data not shown). To determine whether the microinjected Ab-MuLV kinase *per se* was responsible and not a trace contaminating activity, we microinjected partially purified preparations of Ab-MuLV kinase from *E. coli* carrying a gene encoding either an active or an inactive Ab-MuLV kinase. The $^{32}P_i$ content of S6 was increased 15-fold in oocytes microinjected with extracts containing the active kinase relative to oocytes injected with buffer (Fig. 2, lanes 1 and 3). The slight increase (2-fold) in the phosphorylation of S6 in response to injection of the extract containing the inactive Ab-MuLV kinase relative to injection of buffer (Fig. 2, lanes 1 and 2) is probably a nonspecific protein effect because bovine serum albumin also causes a slight increase in S6 phosphorylation (33). This result indicates that the ability to cause S6 phosphorylation is a specific property of the active Ab-MuLV protein kinase because the same *E. coli* proteins were injected in both the control and experimental groups.

The Ab-MuLV-encoded protein kinase specifically phosphorylates tyrosine residues *in vitro* (2, 4); thus, the question of the amino acid acceptor in the S6 protein was important. In both normal NIH 3T3 fibroblasts grown in the presence of serum and ANN-1 cells grown without serum, and also in oocytes induced to undergo maturation by treatment with progesterone or microinjected with highly purified Ab-MuLV kinase, phosphoserine was the only phosphoamino acid detected in S6 (Fig. 3). Furthermore, incubation of 40S ribosomal subunits with [γ-^{32}P]ATP and the Ab-MuLV kinase did not result in phosphorylation of S6 *in vitro* (data not shown). Thus, S6 is probably not a direct substrate for the Ab-MuLV kinase *in vivo*.

To assess the number of phosphate residues incorporated into oocyte S6 in response to microinjection of homogeneous Ab-MuLV kinase, we examined the pattern of electrophoretically separated $^{32}P_i$-labeled S6 derivatives on two-dimensional gels (Fig. 4). In order to detect phosphorylated forms of S6 from control oocytes (in which most of the S6 is in the dephosphorylated form), it was necessary to develop the autoradiograph considerably longer than the autoradiograph of S6 from kinase-injected oocytes. Hence, the large increase in the $^{32}P_i$ content of S6 after microinjection of the Ab-MuLV kinase is not apparent in Fig. 4. In autoradiographs of control (buffer-injected) oocytes, (Fig. 4*B*), the small amount of phosphorylated S6 existed largely in the a, b, and c forms (i.e., 1, 2, and 3 mol of phosphate per mol of S6), whereas after microinjection of the kinase (Fig. 4*A*), forms d and e (i.e., 4 and 5 mol of phosphate per mol of S6) were also visible.

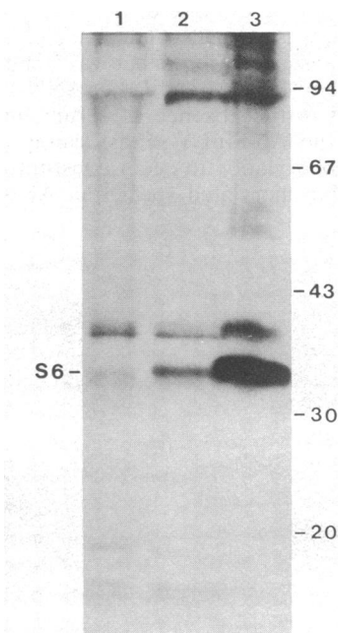

FIG. 2. S6 phosphorylation in oocytes after microinjection of extracts from *E. coli* carrying the Ab-MuLV kinase gene. Oocytes were labeled with $^{32}P_i$ for 4 hr, and Ab-MuLV kinase was partially purified as described. Ribosomes were isolated from equal numbers of oocytes 3 hr after injection, subjected to one-dimensional NaDodSO$_4$ gel electrophoresis on 12.5% gels, and visualized by autoradiography. Lanes: 1, buffer injection; 2, injection of an extract from *E. coli* carrying a transformation- and kinase-deficient mutant; 3, injection of an extract from *E. coli* carrying an active Ab-MuLV kinase. The molecular weights of marker proteins are indicated. The actual cpm in excised S6 bands were 98 (buffer), 231 (inactive extract), and 1476 (active extract).

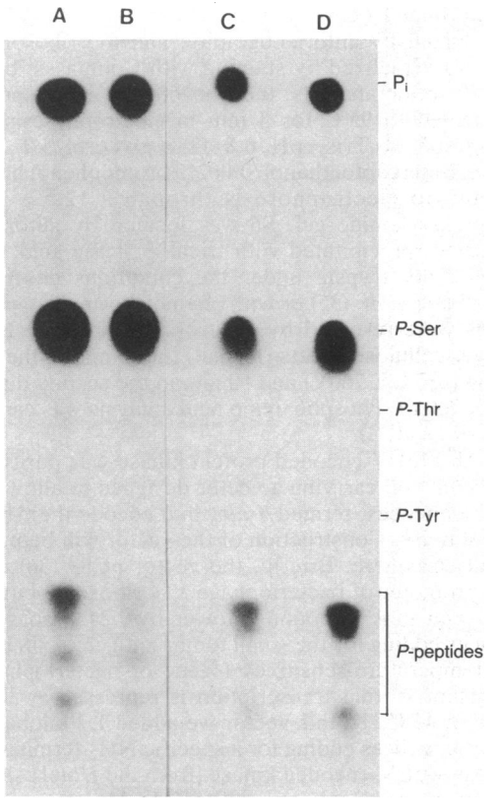

FIG. 3. Phosphoamino acid analysis of phosphorylated S6. ^{32}P-labeled ribosomes were isolated from NIH 3T3 fibroblasts grown in the presence of serum (lane A), from ANN-1 fibroblasts transformed by Ab-MuLV in the absence of serum (lane B), from oocytes treated with 10 μM progesterone (lane C), or oocytes injected with Ab-MuLV kinase (lane D). The ribosomal proteins were separated by electrophoresis through 12.5% NaDodSO$_4$ gels, and the S6 band was located by autoradiography, eluted, hydrolyzed in 6 M HCl at 100°C for 3 hr, and electrophoresed as described. The migration of authentic coelectrophoresed phosphoamino acids was determined by ninhydrin staining.

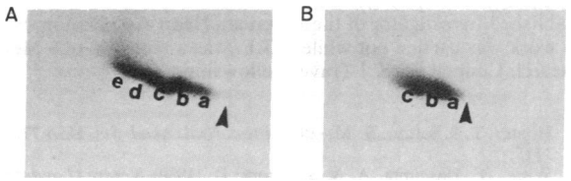

FIG. 4. Two-dimensional gel electrophoresis of S6 from oocytes after microinjection of Ab-MuLV kinase. ^{32}P-labeled oocytes (150 per sample) were injected with either Ab-MuLV kinase (*A*) or buffer (*B*); after 3 hr, ribosomes were isolated, the ribosomal proteins were extracted, and two-dimensional gel electrophoresis was performed as described under *Materials and Methods* except that no carrier ribosomal proteins were added. The arrow marks the position of unphosphorylated S6 as judged from the silver-staining pattern (15, 24). The derivatives a to e refer to S6 species with increasing numbers of phosphate groups. (*A*) Autoradiograph of S6 from oocytes injected with Ab-MuLV kinase. (*B*) Autoradiograph of S6 from buffer-injected oocytes.

To determine whether the sites in S6 phosphorylated as a result of the activity of the Ab-MuLV kinase in oocytes or in ANN-1 cells were the same as in fibroblasts stimulated with serum, we isolated ribosomes, labeled as in Figs. 1 and 4, and separated their proteins by 1-dimensional gel electrophoresis. S6 was eluted and then treated with either trypsin or chymotrypsin and analyzed by two-dimensional thin-layer high-voltage electrophoresis. Previous studies of S6 phosphopeptides have utilized tryptic digests (35); but we find that the use of chymotrypsin results in a more reproducible pattern. The phosphopeptide maps of S6, with either trypsin or chymotrypsin, were identical, regardless of whether S6 was isolated from normal cells in the presence of serum (Fig. 5 *A* and *C*) or from Ab-MuLV-transformed cells in the absence of serum (Fig. 5 *B* and *D*). The phosphopeptide map of S6 from oocytes microinjected with Ab-MuLV kinase was equivalent to the pattern obtained with S6 from cells transformed by Ab-MuLV (Fig. 5*E*); moreover, this map revealed new phosphopeptides not apparent in control oocytes (Fig. 5*F*). Recently Blenis *et al.* (39) have demonstrated similar phosphopeptides in S6 isolated from either RSV-transformed or serum-stimulated chicken embryo fibroblasts.

DISCUSSION

It is evident that *Xenopus* oocytes can respond to a foreign transforming gene product by an increased phosphorylation of ribosomal protein S6. Microinjection of a homogeneous preparation of the Ab-MuLV kinase into oocytes resulted in a 7- to 15-fold increase in the $^{32}P_i$ content of S6 and the formation of S6 derivatives containing 4 and 5 mol of phosphate per mol of S6. These results in oocytes support the idea that S6 phosphorylation as observed in Ab-MuLV-transformed cells in the absence of serum is a direct consequence of the activity of the Ab-MuLV gene product. The data indicating that an Ab-MuLV protein that is kinase-defective is unable to increase S6 phosphorylation in oocytes are consistent with the hypothesis that the tyrosine kinase activity of the Ab-MuLV gene product is responsible for the stimulation of S6 phosphorylation. We cannot rule out, however, the possibility that other as yet unidentified activities of the Ab-MuLV gene product are responsible. The normal Ab-MuLV gene product is a fusion protein containing NH_2-terminal sequences derived from the *gag* region of Mo-MuLV and sequences derived from the cellular c-*abl* gene, which encode the tyrosine kinase activity (40). The Ab-MuLV protein used

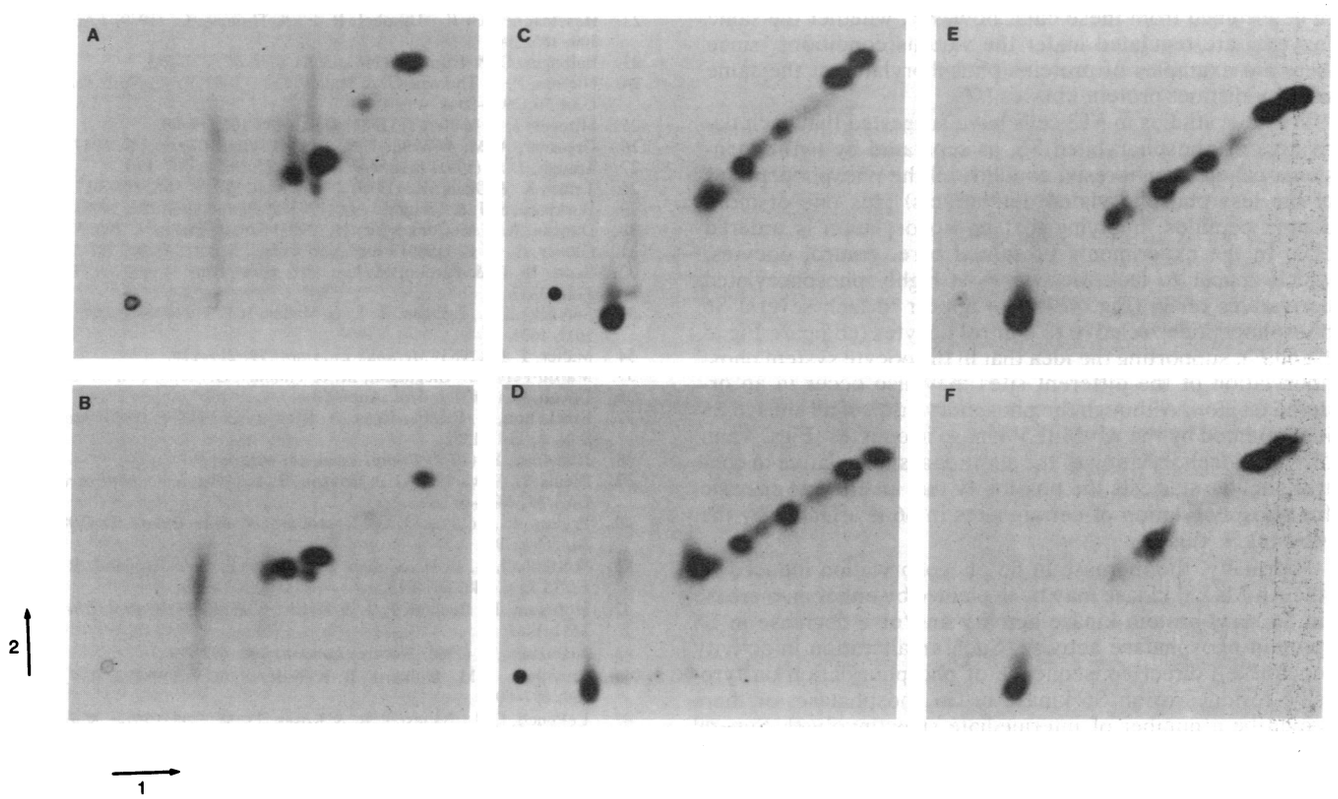

FIG. 5. Phosphopeptide analysis of S6. ^{32}P-labeled ribosomes were isolated from NIH 3T3 fibroblasts labeled in the presence of 10% calf serum, from Ab-MuLV-transformed cells (ANN-1) grown in the absence of serum, or from oocytes injected with Ab-MuLV kinase. The S6 was isolated and digested, and the phosphopeptides were resolved as described. (*A*) Tryptic pattern from NIH 3T3 cells with 10% serum. (*B*) Tryptic pattern from ANN-1 cells in absence of serum. (*C*) Chymotryptic phosphopeptide pattern from NIH 3T3 cells in the presence of 10% serum. (*D*) Chymotryptic pattern from ANN-1 cells in the absence of serum. (*E*) Chymotryptic pattern of oocyte S6 3 hr after Ab-MuLV kinase injection. (*F*) Chymotryptic pattern of oocyte S6 from control oocytes.

in these studies lacks the *gag* region and instead has 80 amino acids derived from the small tumor antigen of simian virus 40. Although this protein clearly retains an active tyrosine kinase activity, the ability of this particular protein to transform cells has not been investigated, and at least some of the *gag* region is needed for efficient transformation of lymphoid cells by Ab-MuLV (40).

The stimulation of S6 phosphorylation in oocytes by the Ab-MuLV kinase occurred at an intracellular kinase concentration of about 50 nM, if one assumes 1 μl of cell water. Although we do not know the concentration of the Ab-MuLV kinase in virally transformed cells, the intracellular concentration of the cyclic AMP-dependent protein kinase, which has been shown to phosphorylate S6 directly in response to elevation of cyclic AMP (35, 41), is $\approx$1 μM (42).

The results of phosphopeptide mapping with either trypsin or chymotrypsin suggest that microinjected Ab-MuLV kinase induced the phosphorylation of *Xenopus* S6 at sites that are identical to those in S6 isolated from ANN-1 cells in the absence of serum or isolated from NIH 3T3 cells in the presence of serum (Fig. 5). This indicates that S6 must be highly conserved in eukaryotic cells. Treatment of fibroblasts with the tumor promoter phorbol ester, which is known to activate protein kinase C and induce S6 phosphorylation (43–45), also results in phosphorylation of S6 at identical phosphopeptides (ref. 39; also our data, not shown). These analyses indicate that there are no subclasses of sites uniquely associated with the action of a single growth-promoting agent. This conclusion is in agreement with that of Martin-Perez *et al.* (35), who observed that treatment of Swiss mouse 3T3 cells with either epidermal growth factor, insulin, or prostaglandin $F_2\alpha$ to cause partial S6 phosphorylation, induces an identical pattern of S6 phosphopeptides. It cannot be determined from these data, however, whether the same enzymes are regulated under the various conditions, since there are examples of proteins phosphorylated at the same sites by distinct protein kinases (46).

Previous studies in 3T3 cells have suggested that each derivative of phosphorylated S6, as separated by two-dimensional gel electrophoresis, contains all the phosphopeptides of the less phosphorylated derivative(s) plus one or more unique peptides, implying that phosphorylation is ordered (35). In the experiments presented here, control oocytes, which appear to lack the two most highly phosphorylated derivatives of S6 (Fig. 4*B*), also appear to lack several S6 phosphopeptides relative to control oocytes (compare Fig. 5 *E* and *F*), supporting the idea that in the oocyte system phosphorylation of the different sites may also occur in an ordered fashion. Although the phosphorylation of all sites in S6 is stimulated by the Ab-MuLV kinase in oocytes (Figs. 4 and 5), the phosphorylation of the additional sites relative to control oocytes suggests the possibility that an enzyme specific for phosphorylation of certain sites in S6 is affected by the Ab-MuLV kinase.

Formally, the increase in S6 phosphorylation induced by the Ab-MuLV kinase may be explained by either an increase in S6 seryl-protein kinase activity and/or a decrease in S6 protein phosphatase activity. Such an alteration in activity could be a direct consequence of phosphorylation on tyrosine residues of an S6 kinase or S6 phosphatase, or there could be a number of intermediate steps involved. Several S6 protein kinases have been described (29, 41, 45, 47–50) that are potential substrates for a direct effect of the Ab-MuLV kinase.

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1. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
2. Witte, D., Dasgupta, A. & Baltimore, D. (1980) *Nature (London)* **283**, 826–831.
3. Collett, M. S., Purchio, A. F. & Erikson, R. L. (1980) *Nature (London)* **285**, 167–169.
4. Wang, J. Y. J., Queen, C. & Baltimore, D. (1982) *J. Biol. Chem.* **257**, 13181–13184.
5. Feldman, R., Wang, L. H., Hanafusa, H. & Balduzzi, P. C. (1982) *J. Virol.* **42**, 228–236.
6. Naharro, G., Dunn, C. Y. & Robbins, K. C. (1983) *Virology* **125**, 502–507.
7. Feldman, R. A., Hanafusa, T. & Hanafusa, H. (1980) *Cell* **22**, 757–765.
8. Kawai, S., Yoshida, M., Segawa, K., Sugiyama, H., Ishizaki, R. & Toyoshima, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6199–6203.
9. Neil, J. C., Ghysdael, J. & Vogt, P. K. (1981) *Virology* **109**, 223–228.
10. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363–8365.
11. Ek, B., Westermark, B., Wasteson, A. & Heldin, C.-H. (1982) *Nature (London)* **295**, 419–420.
12. Rubin, J. B., Shia, M. A. & Pilch, P. F. (1983) *Nature (London)* **305**, 438–440.
13. Jacobs, S., Kull, F. C., Earp, H. S., Svoboda, M. E., Van Wyk, J. J. & Cuatrecasas, P. (1983) *J. Biol. Chem.* **258**, 9581–9584.
14. Kasuga, M., Zick, Y., Blithe, D. L., Crettaz, M. & Kahn, C. R. (1982) *Nature (London)* **298**, 667–669.
15. Thomas, G., Martin-Perez, J., Siegmann, M. & Otto, A. M. (1982) *Cell* **30**, 235–242.
16. Nishimura, J. & Deuel, T. F. (1981) *Biochem. Biophys. Res. Commun.* **103**, 355–361.
17. Kasuga, M., Zick, Y., Blith, D. L., Karlsson, F. A., Haring, H. U. & Kahn, C. R. (1982) *J. Biol. Chem.* **257**, 9891–9894.
18. Swergold, G. D., Rosen, O. M. & Rubin, C. S. (1982) *J. Biol. Chem.* **257**, 4207–4215.
19. Cooper, J. A. & Hunter, T. (1983) *J. Biol. Chem.* **258**, 1108–1115.
20. Decker, S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4112–4115.
21. Nishimura, J. & Deuel, T. F. (1983) *FEBS Lett.* **156**, 130–134.
22. Haselbacher, G. K., Humbel, R. E. & Thomas, G. (1979) *FEBS Lett.* **100**, 185–190.
23. Ballinger, D. & Hunt, T. (1981) *Dev. Biol.* **87**, 277–285.
24. Nielsen, P. J., Thomas, G. & Maller, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2937–2941.
25. Stith, B. J. & Maller, J. (1984) *Dev. Biol.* **102**, 79–89.
26. Gressner, A. M. & Wool, J. G. (1974) *J. Biol. Chem.* **249**, 6917–6925.
27. Traugh, J. A. (1981) *Biochem. Actions Horm.* **8**, 167–179.
28. Terao, K. & Ogata, K. (1979) *J. Biochem. (Tokyo)* **86**, 605–617.
29. Burkhard, S. J. & Traugh, J. A. (1983) *J. Biol. Chem.* **258**, 14003–14008.
30. Duncan, R. & McConkey, E. H. (1982) *Eur. J. Biochem.* **123**, 535–538.
31. Glover, C. V. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1781–1785.
32. Scott, M. P. & Pardue, M. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3353–3357.
33. Spivack, J. G., Erikson, R. L. & Maller, J. L. (1984) *Mol. Cell. Biol.* **4**, 1631–1634.
34. Maller, J. L. (1983) *Methods Enzymol.* **99**, 219–226.
35. Martin-Perez, J., Siegmann, M. & Thomas, G. (1984) *Cell* **36**, 287–294.
36. Queen, C. (1983) *J. Mol. Appl. Genet.* **2**, 1–10.
37. Frackelton, A. R., Jr., Ross, A. H. & Eisen, H. N. (1983) *Mol. Cell. Biol.* **3**, 1343–1352.
38. Hanafusa, H. (1977) *Comp. Virol.* **10**, 401–481.
39. Blenis, J., Spivack, J. G. & Erikson, R. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6408–6412.
40. Prywes, R., Foulkes, J. G., Rosenberg, N. & Baltimore, D. (1983) *Cell* **34**, 569–579.
41. Wettenhall, R. E. H., Cohen, P., Caudwell, B. & Holland, R. (1982) *FEBS Lett.* **148**, 207–213.
42. Hofmann, F., Bechtel, P. J. & Krebs, E. G. (1977) *J. Biol. Chem.* **252**, 1441–1447.
43. Nishizuka, Y. (1984) *Nature (London)* **308**, 693–698.
44. Trevillyan, J. M., Kulkarni, R. K. & Byus, C. V. (1984) *J. Biol. Chem.* **259**, 897–902.
45. LePeuch, C. J., Ballester, R. & Rosen, O. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6858–6862.
46. Parker, P. J., Caudwell, F. B. & Cohen, P. (1983) *Eur. J. Biochem.* **130**, 227–234.
47. Perisic, O. & Traugh, J. A. (1983) *J. Biol. Chem.* **258**, 9589–9592.
48. Cobb, M. H. & Rosen, O. M. (1983) *J. Biol. Chem.* **258**, 12472–12481.
49. Donahue, M. J. & Masaracchia, R. A. (1984) *J. Biol. Chem.* **259**, 435–440.
50. Novak-Hofer, I. & Thomas, G. (1984) *J. Biol. Chem.* **259**, 5995–6000.